

Oviposition site selection by *Coenagrion mercuriale* (Odonata: Coenagrionidae)

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ABSTRACT

The aim of the study was to determine oviposition site selection in the endangered damselfly *Coenagrion mercuriale* in its UK stronghold and to determine hatching success of eggs. This was achieved by watching the behaviour of marked pairs from the onset of copulation to the end of oviposition and recording the number and duration of oviposition attempts and the plants oviposited in during the pairing. Pairs were either freely observed along a stream or placed in pre-placed cages within the stream. Stems into which oviposition had been observed were collected after four weeks and the fate of deposited eggs was determined. Pairs typically oviposited in several stems during multiple oviposition bouts, but usually in just one plant species. Mean total duration of oviposition behaviour was 671 s but ranged from 244 to 1,471 s. Mean number of eggs laid was 91 and ranged from 23 to 337. The female submerged completely in 15% of ovipositions. Mean egg deposition rate was 14 eggs per min but there was considerable variation. There was a significant positive relationship between total duration of oviposition in a stem and number of eggs laid in that stem. None of the habitat variables measured was a good predictor of duration of oviposition or number of eggs laid. Mean mortality of eggs was 14% at the time of collection and there was asynchronous development. *Hypericum elodes* was used significantly more than expected from its frequency in the environment and *Eleocharis palustris*, *Molinia caerulea* and *Myrica gale* significantly less.

INTRODUCTION

Habitat is an autecological concept that emphasises the interaction between a species and the physical structure of its environment (Samways 1994). Organisms must select habitat that meets the ecological needs of all the stages of their life cycle and habitat use is determined by selection on survival and reproduction, arising from differential fitnesses between habitats (Boyce & McDonald 1999). Investigation of the proximate cues to which animals respond during habitat selection can help to

elucidate features of the habitat that are required by the species (ultimate factors). These cues may have either a direct or indirect relationship with such habitat features i.e. either they are the features required or they indicate the presence of those features. In this paper we investigate the selection of habitat for oviposition by the endangered damselfly *Coenagrion mercuriale* (Charpentier) in its UK stronghold.

Some adult odonates home to the water body from which they emerged (Utzeri et al. 1984) but most carry out active habitat selection (Corbet 1999:17-21). Wildermuth (1994) suggested that odonates respond to features of their habitat in a hierarchical manner. Buchwald (1994a, b) focussing on *Ceriagrion tenellum* (de Villers) also adopted a four-step model for habitat choice. Wildermuth's scheme follows. Adults respond to the biotope at a large scale when flying at decimetres above the habitat (e.g. wet heathland/valley mire for *C. mercuriale* in the UK). At 0.5-5 m above the habitat, they respond to features of the larval water body (small stream or runnel for *C. mercuriale*), whilst at 0-0.5 m above the water they respond to the features of the oviposition site. More general cues are likely to be detected visually and tactile or thermosensory cues will be used only at the final stages of the hierarchy. This study investigates the cues used by *C. mercuriale* at this final stage by measuring the utilisation of a stem for oviposition once a pair has landed. Site utilisation for oviposition is then related to a variety of habitat variables that may be used as proximate cues and thus may relate to ecological requirements.

Females may select habitat features that minimise the costs of oviposition itself as well as attempting to maximise the subsequent benefits to their offspring. These costs may be the energy expended during oviposition, the loss of time and energy due to harassment by male conspecifics (Ubukata 1984; Waage 1987), predation (Fincke 1986; Michiels & Dhondt 1990) and avoidance of egg parasitoids (A. Cordero Rivera pers. comm.).

Previous oviposition habitat selection experiments have focused on territorial (Ubukata 1984; Waage 1987; Meek & Herman 1991; Wingfield Gibbons & Pain 1992; Tsubaki et al. 1994; Siva-Jothy et al. 1995), rather than non-territorial odonate species (Martens 1989, 1992, 1999; Michiels & Dhondt 1990; Rehfeldt 1990). In the former, oviposition does not usually occur in tandem and males guard patches of suitable oviposition habitat and, in doing so, gain access to mates. Thus female choice of oviposition location is likely to be influenced strongly by the location and behaviour of conspecifics as well as by oviposition site quality. *C. mercuriale* in contrast is non-territorial and oviposition usually occurs in tandems which are rarely broken up by conspecific males and thus oviposition site quality is likely to have an overriding influence on oviposition habitat selection.

Whilst this study focuses on microhabitat use by *C. mercuriale*, the aspects of a pair's oviposition behaviour that may be influenced by the species ecological requirements were also observed. To investigate how cues used for oviposition relate to the ecological requirements of the egg stage, egg-hatching success was measured in stems from focal watches, which were defined as pairs watched continuously from copulation to the end of oviposition, carried out in natural conditions. Most studies have estimated egg mortality and larval mortality together or have tried to estimate egg mortality from the difference between egg input and density of early instar larvae (Macan 1964; Lawton 1970). Since the latter are notoriously difficult to sample it is better to examine egg mortality from plant material left in situ until after the hatch period (Bennett & Mill 1995). In this study, this technique was developed in an attempt to quantify possible sources of egg mortality in *C. mercuriale*.

Identifying the common features that constitute ecological requirements of a species and which cues are used by individuals to recognise them is particularly important in a rare species such as *C. mercuriale*. The availability of such features or cues may determine the distribution of adults between habitats within a site and may influence the distribution of populations across a species range. In addition, *C. mercuriale* occupies different habitats in different parts of mainland Europe (Buchwald 1989) and the UK and it is interesting to consider to what extent ecological requirements differ in different parts of its range.

METHODS

Oviposition site selection

The study was carried out on a large adult population of *Coenagrion mercuriale* on a stream running through wet heathland at Upper Crockford, New Forest, southern England (50.796°N, 1.509°W). Individuals had been marked by writing a number on the left fore wing with waterproof ink. Focal watches were made on marked ovipositing pairs and the duration and number of oviposition bouts on each stem was recorded as a measure of utilisation of an oviposition site.

Two types of focals were carried out. Natural focals were carried out when pairs came to the stream to oviposit after copulation. Stems into which eggs had been laid were uniquely marked with coloured plastic pegs for later retrieval and covered with wire to prevent subsequent oviposition. However, since many ovipositions would have occurred on the site before the study began, it was conceivable that eggs could have been laid by previous pairs into stems used by focal pairs, potentially making it difficult to attribute eggs to a particular pair. Thus a series of caged focals were carried out by placing pairs found in tussocks, in copula or before copulation, into cages that had been in place since the beginning of the flight period and contained vegetation that had not been used previously for oviposition. Each cage covered an area of 0.72 m² and consisted of a light, triangular, tent frame covered by transparent, fine mesh netting (see Purse et al. 2003a for detailed methods). Stems were removed immediately after the focal watch for dissection ensuring data on egg deposition rate, whereas some stems from natural focals were not recovered until after the hatch period. Caged focals also permitted the entire oviposition period of pairs to be observed without disturbance. The time of day and temperatures (water and air) were recorded during each bout of each focal.

Habitat variables

For each stem that had been selected for oviposition, the following characteristics were recorded: species, length of stem above water, size of surrounding vegetation patch, coded as follows: 1 = single stem, 2 = small patch with two to 10 stems, 3 = large patch with more than 10 stems, the distance of the stem from the nearest discernible area of flow and from the nearest sheltering tussock. In addition the vegetation communities were characterised by estimating percentage cover of plant species within 1m x 1m quadrats around each oviposition site. For comparison, background stream habitat was characterised by random placement of quadrats along the stream. Tablefit version 1.0 (Hill 1989) was used to classify the quadrat samples of vegetation into the National Vegetation Community classifications (Rodwell 1991) and the European Community Corine System.

Egg hatching success

Marked stems from natural focals were removed after four weeks, one week longer than the hatching period in captivity (Corbet 1955). Either after egg deposition (caged stems) or after the hatch period (natural stems), the plant material retrieved was put into tap water for 2-6 days, transferred to a mixture of concentrated acetic acid and 96% ethanol in a 2:1 ratio for 24 hours, cleaned in 96% ethanol before being preserved until dissection in 70% ethanol. Stems were dissected to remove the eggs, using micropyles protruding from the leaf surface as a guide to their location, and their size and appearance were used to grade their stage of development (after Waage 1978). Thus for each stem, the total number of eggs, the total duration of oviposition into a stem, the number of eggs in each developmental stage (after hatch period only), the plant species, the stem diameter (mean from five measurements of the area containing eggs), the total stem length and the length of stem covered by eggs, were recorded.

Statistical analysis

Two sample *t*-tests were performed to check that the focal pair's environment (i.e. caged or natural) had no influence on oviposition behaviour. A chi-squared test was used to determine whether focal type influenced the number of different plant species used by a pair. Site utilisation for oviposition was quantified at the level of the stem. Quantitative effects of habitat variables on utilisation of oviposition sites was evaluated with multiple stepwise regression and logistic models with total duration of oviposition (square-root transformed) and number of eggs laid on a stem (square-root transformed) across all bouts on a stem as the dependent variables. In other zygoterans, eggs are not deposited during short initial periods of abdominal contact e.g. ca 40 s in *Platycnemis pennipes* (Pallas) (Martens 1992). The duration of these non egg-laying 'test' bouts in *C. mercuriale* can be predicted from the y-intercept of a regression of the total duration of oviposition against the number of eggs laid (see results) for stems with only one oviposition bout.

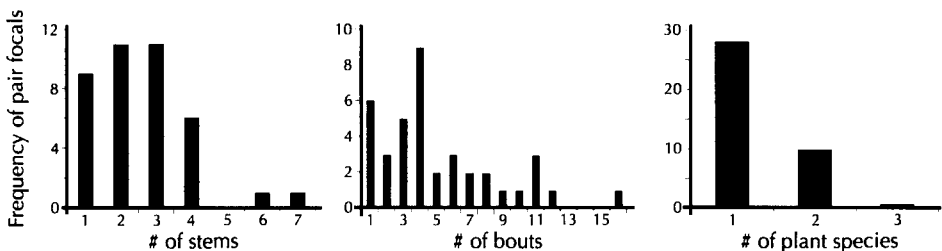


Figure 1: The use of different plant species, number of plant stems and number of oviposition bouts by observed pairs of ovipositing *Coenagrion mercuriale* — frequency distribution of (a) stems used; (b) bouts of oviposition carried out; and (c) different plant species used.

Habitat predictors were air temperature 10 cm above water surface, water temperature 5 cm below water surface, pH, water depth, distance from discernible flow (square-root transformed), distance from nearest tussock (i.e. shelter), length of emergent stem (square-root transformed), diameter of stem. For logistic models, three coded binary variables were created as to whether the duration of oviposition exceeded 50, 100 or 195 s, the last being the median duration of oviposition. Preferred plant species for oviposition were investigated through the use of χ^2 tests, to test whether plant species were used more or less frequently than expected from their proportional abundance levels in the study stream.

RESULTS

General oviposition behaviour

Due to the difficulty of tracking pairs of *Coenagrion mercuriale* over wet heath, it was possible to observe the entire oviposition period – from copulation until the pair split up – in only 39 (19 caged and 20 natural) out of 80 focals attempted and the subsequent analysis was restricted to these. After copulation in tussocks next to the stream, pairs flew towards the stream and landed repeatedly on plant substrates. In the initial phase, females would frequently ‘probe’ stems with their ovipositors several times – with contact between stem and ovipositor lasting for less than 5 s. Thus, an oviposition bout was deemed to have begun when the period of contact between the female’s ovipositor and the plant stem exceeded 5 s. Regardless of whether they were observed in caged or natural focals, pairs usually oviposited into 1 to 4 stems ($m \pm \text{s.e.}_{\text{caged}} = 2.80 \pm 0.38$, $m \pm \text{s.e.}_{\text{natural}} = 2.37 \pm 0.90$, $t = 0.997$, $p = 0.33$, d.f. = 37), performing 5 to 6 bouts of oviposition ($m \pm \text{s.e.}_{\text{caged}} = 5.70 \pm 0.92$, $m \pm \text{s.e.}_{\text{natural}} = 4.58 \pm 0.69$, $t = 0.965$, $p = 0.34$, d.f. = 37) but generally used only one particular plant species ($\chi^2 = 2.82$, 1 d.f., $p = 0.093$) (Fig. 1).

Oviposition behaviour lasted for a total duration of 11.18 ± 0.82 min ($n = 36$) on average, ranging from 4.06 to 24.51 min with no significant difference in duration between caged and natural focals ($m \pm \text{s.e.}_{\text{caged}} = 10.83 \pm 0.93$, $m \pm \text{s.e.}_{\text{natural}} = 11.54 \pm 1.39$, $t = -0.426$, $p = 0.67$, d.f. = 37). Pairs laid 90.5 ± 22.3 eggs on average, ranging from 23 to 337 eggs ($n = 13$ due to frequent loss/damage of stems in stream before retrieval). The female submerged to oviposit always up to her wings but submerged completely in 15.4% (6/39) of ovipositions. When the female submerged completely the male broke the tandem and flew to the nearest perch above the water where oviposition was taking place. The male usually left before oviposition was completed. In three focals, oviposition was terminated when the female was caught in *Drosera rotundifolia*. Only 5% (2/39) of *C. mercuriale* pairs were predated upon during oviposition, one by a water strider and one by a water spider.

Measures of site utilisation for oviposition

Forty-seven stems were used during the 19 caged focals and 62 stems were used during 20 natural focals. There was no difference between stems from caged and natural focals with regards to total number of bouts into each stem ($\chi^2 = 5.58$, 2 d.f., $p = 0.061$), total duration of oviposition (Wilcoxon two sample test; median_{caged} = 195.0, $n_{\text{caged}} = 45$; median_{natural} = 178.0, $n_{\text{natural}} = 60$; $W = 2,986.0$, $p = 0.209$) and

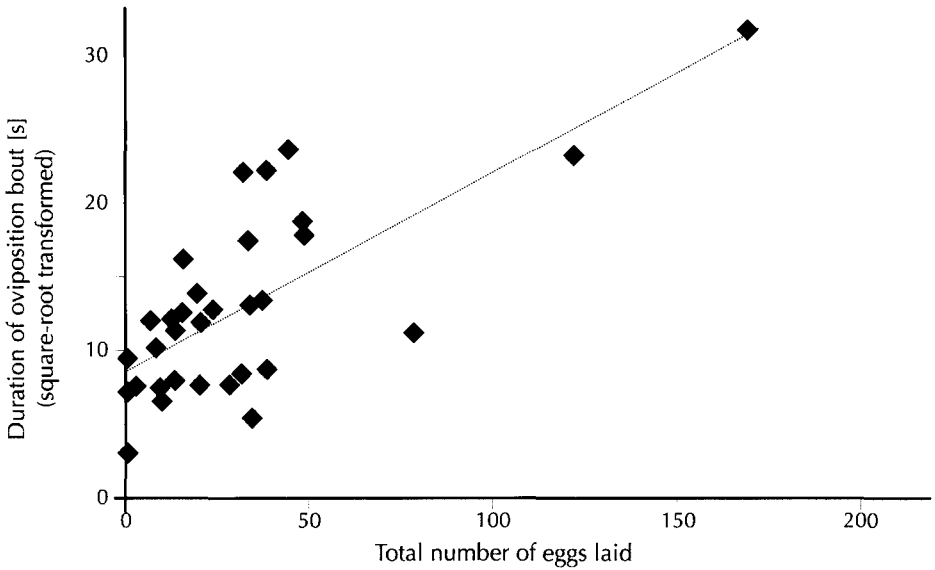


Figure 2: Length of oviposition bout and the number of eggs laid by *Coenagrion mercuriale* — relationship between duration of an oviposition bout on a stem (in seconds) and the total number of eggs laid into it – for stems where only one bout of oviposition was recorded.

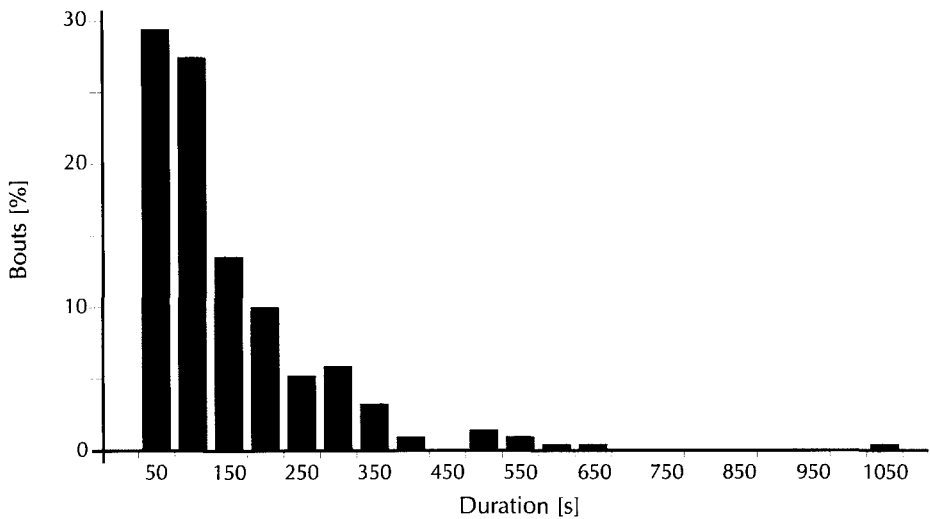


Figure 3: Percentage distribution of duration for all oviposition bouts observed in *Coenagrion mercuriale*; $n = 207$; bar labels indicate the upper limit of the class interval.

Table 1. Use of plant species for oviposition of *Coenagrion mercuriale* — χ^2 test of difference between observation (O) and expectancy (E).

Plant species	Frequency of stems from					
	Caged focals			Natural focals		
	E	O	(O-E) ² /E	E	O	(O-E) ² /E
<i>Eleocharis palustris</i>	7	0	7.00	23	0	23.00
<i>Hypericum elodes</i>	29	44	7.76	9	30	49.00
<i>Juncus articulatus</i>	2	1	0.50	2	5	4.50
<i>Molinia caerulea</i>	2	0	2.00	5	1	3.20
<i>Myrica gale</i>	4	0	4.00	11	0	11.00
<i>Potamogeton polygonifolius</i>	-	-	-	6	21	37.50
			$\chi^2 = 21.26$			$\chi^2 = 128.20$

total number of eggs laid (Wilcoxon two sample test; median_{caged} = 31.0, $n_{\text{caged}} = 40$; median_{natural} = 28.5, $n_{\text{natural}} = 22$; $W = 1,209.0$, $p = 0.453$). Thus both caged and natural stems could be amalgamated for subsequent analysis. Mean total duration of oviposition on a stem was 247.3 ± 21.9 s ($n = 195$) and ranged from 10 to 1,073 s. Mean total number of eggs laid on a stem was 44.12 ± 8.39 eggs ($n = 30$) and ranged from 0 to 353 eggs. Mean deposition rate, calculated from 29 stems into which one bout of oviposition occurred, was 14.06 ± 2.66 eggs per min and ranged from 3 to 65.81. Figure 2 shows the value of the y intercept to be 8.819 with a 95% confidence interval of between 6.738 and 10.90. The duration of non egg-laying 'test' bouts is predicted to be 77.8 s with a 95% confidence interval of between 45.4 and 118.8 s. Although 30% of all oviposition bouts observed were less than 50 s, i.e. near the lower limit of the 95% confidence interval (Fig. 3), suggesting that many bouts are non-egg laying 'test' bouts. A significant positive relationship was observed between total duration of oviposition on a stem and total number of eggs laid on a stem (Fig. 4) indicating that total duration of oviposition could be used as measure of site suitability for oviposition where total number of eggs could not be estimated for a stem, due to decomposition or loss. The variance explained in total number of eggs laid on a stem was not improved if the total duration of oviposition on a stem was corrected by removal of short, non-egg laying test bouts so uncorrected results are presented here.

Effect of habitat variables on site utilisation for oviposition

No quantitative effects of continuous habitat variables could be detected either on the total duration of oviposition per stem or on the number of eggs laid on a stem. Qualitatively, oviposition behaviour occurred at air temperatures of between 13.8 and 28.5°C ($m = 23.05 \pm 0.36$) and at water temperatures of between 18.1 and 29.9 ($m = 24.96 \pm 0.30$). The pH of water in which oviposition occurred ranged from 5.5 to 7.9 ($m = 6.67 \pm 0.05$). Whilst the depth of the water in which oviposition occurred ranged from 1.5 to 25 cm ($m = 8.19 \pm 0.55$), 75% of ovipositions occurred at depths of 9 cm or below. The distance from discernible water flow ranged from 0 to 4 m ($m = 62.1 \pm 10.1$) but 75% of ovipositions occurred within 80 cm of flow.

The distance from the nearest tussock ranged from 0 to 2 m ($m = 61.4 \pm 6.6$). In addition oviposition always occurred on areas of stream with a dark brown peat rather than gravel substrate. With regards to the features of the stem itself, oviposition occurred in stems with diameters of between 0.74 and 4.78 mm ($m = 1.99 \pm 0.10$) and, in 75% of stems, only 4 cm of the stem protruded above the surface of the water ($m = 2.91 \pm 0.37$). The maximum distance (below water) between where eggs were placed on the stem and the surface of the water ranged from 0.9 cm to 8.7 cm ($m = 3.8 \pm 0.54$, $n = 16$), reflecting the distances attained when females submerge to oviposit. The distance along the stem covered by the egg clutch ranged from between 3 mm to 52 mm ($m = 18.7 \pm 0.17$, $n = 38$). Forty-three per cent of stems used for oviposition occurred singly whilst 28% were positioned within a small patch (2-10 stems) and 29% were positioned within a large patch of vegetation (> 10 stems, $n = 97$).

Egg hatching success

After four weeks, mean mortality rate of eggs was $14\% \pm 6\%$. $65\% \pm 10\%$ of eggs had hatched on average, 21% were healthy but in stages of development before hatching. No parasitoids were detected. This suggests that the hatch period of eggs can last considerably longer than four weeks in their natural environment. Due to the low sample size of stems it was not possible to investigate, in detail, the effect of habitat variables on egg hatching success.

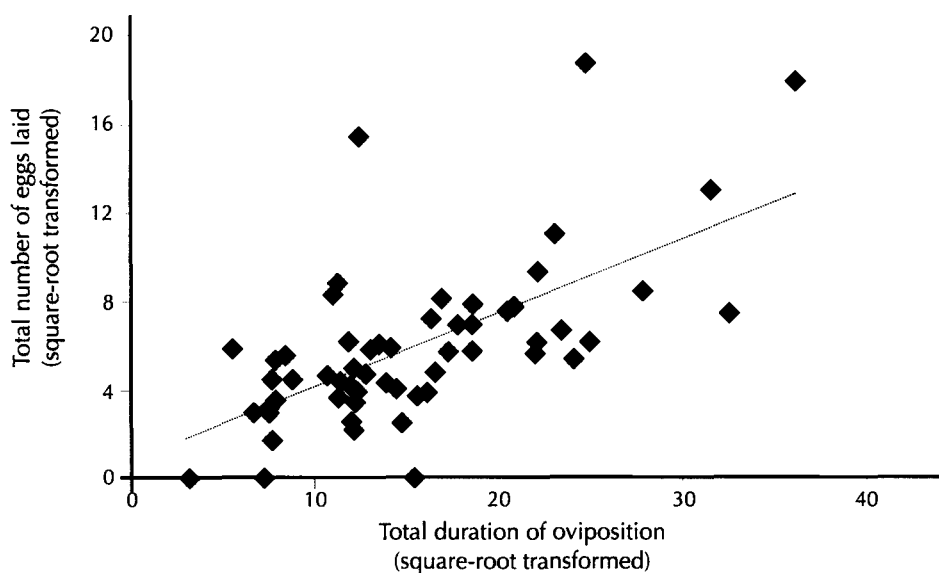


Figure 4: Relationship between total numbers of eggs laid and total duration of oviposition on a stem (all stems) by pairs of *Coenagrion mercuriale*.

Table 2. Plant species composition and plant use for oviposition of *Coenagrion mercuriale* — comparison between natural habitat and cages. M_{cov} : mean % cover of plant species; FS_O : observed frequency of stems; FS_e : expected frequency of stems.

Plant species	Cages			Natural habitat		
	M_{cov}	FS_O	FS_e	M_{cov}	FS_O	FS_e
<i>Anagallis tenella</i>	0.6	0	0	< 0.1	0	0
<i>Carex panicea</i>	< 0.1	0	0	2.4	3	1
<i>Cirsium dissectum</i>	2.1	0	1	1.2	1	1
<i>Drosera rotundifolia</i>	1.1	1	0	0.6	0	1
<i>Eleocharis palustris</i>	14.2	0	7	37.7	0	22
<i>Erica tetralix</i>	0.4	0	0	1.2	0	1
<i>Eriophorum</i> sp.	< 0.1	0	0	< 0.1	0	0
<i>Hypericum elodes</i>	61.2	44	27	14.1	30	8
<i>Juncus articulatus</i>	4.8	1	2	3.5	5	2
<i>Mentha aquatica</i>	< 0.1	0	0	< 0.1	1	0
<i>Molinia caerulea</i>	3.3	0	2	8.8	1	5
<i>Myrica gale</i>	8.2	0	3	17.7	0	11
<i>Narthecium ossifragum</i>	< 0.1	0	0	2.4	0	1
<i>Pedicularis</i> sp.	1.1	0	0	0.6	0	0
<i>Potamogeton polygonifolius</i>	2.2	1	1	9.8	21	7
<i>Ranunculus flammula</i>	0.8	0	0	< 0.1	0	0
<i>Rhynchospora alba</i>	0.2	0	0	< 0.1	0	0

Use of plant species and communities for oviposition

C. mercuriale used the following plant species (and parts) for oviposition at Upper Crockford; *Carex panicea* (petiole), *Cirsium dissectum* (stem), *Drosera rotundifolia* (stem), *Hypericum elodes* (stem), *Juncus articulatus* (stem), *Mentha aquatica* (stem), *Molinia caerulea* (stem), *Potamogeton polygonifolius* (petiole and central leaf surface). In caged focals 47 stems of four plant species were utilised. The availability of plant species in the study stream is shown in Table 2 for cages and natural focals separately and can be compared with the frequency of use of plant species for oviposition in Table 2. For species for which more than two stems were used across caged focals, *H. elodes* was used more often than expected from its abundance in cages whilst *Eleocharis palustris*, *Molinia caerulea* and *Myrica gale* were used less often than expected ($\chi^2 = 21.26$, 4 d.f., $p < 0.001$; Table 1). These results were mirrored by stem use in natural focals, though *P. polygonifolius* was additionally used for oviposition more often than expected from its abundance ($\chi^2 = 128.20$, 5 d.f., $p < 0.001$, Table 1). Though it was not used for egg-laying, the percentage cover of *M. gale* at 15% ($n = 25$) was higher in areas surrounding stems selected for oviposition in natural focals compared to its background cover of 4% ($n = 22$) in stream quadrats (Wilcoxon $W = 380$, $p < 0.002$). The most common NVC plant community in both oviposition and stream quadrats was S19, or *E. palustris* swamp.

DISCUSSION

In this study the proximate cues used by *Coenagrion mercuriale* to select habitat for oviposition were identified, focusing on the final stage of the hierarchy proposed by Wildermuth (1994) and Buchwald (1994a). The proximate cues used at the smallest scale by *C. mercuriale* are compared with information on the habitat features required within site and between sites by this species. In particular, phytosociological studies on odonates have focused on the relationship between species distribution and aspects of the vegetation structure at sites (for *C. mercuriale* see Buchwald 1983, 1989, 1992, 1994c; Buchwald et al. 1989; Röske 1995).

General oviposition behaviour

Pairs usually oviposited in several stems with several bouts of oviposition on each stem (cf. Rehfeldt 1990; Tsubaki et al. 1994; Bennett & Mill 1995). Most pairs used stems of the same plant species for all bouts. Whilst, changes in position within and between stems could be a strategy for distributing eggs between several sites, especially in populations which are heavily predated either during the egg stage or during oviposition (Buskirk & Sherman 1984; Rehfeldt 1990), they most probably occurred in *C. mercuriale* after unsuccessful attempts at oviposition.

In *C. mercuriale*, as in some other zygopterans (Okazawa & Ubukata 1978; Martens 1992), eggs were not deposited during short initial periods of abdominal contact with a stem. These non egg-laying bouts were predicted to last between 45 and 118 s (95% confidence intervals). Almost 30% of all oviposition bouts observed during focals were less than 50 s in duration and probably did not involve egg laying. Similarly, in a French population of *C. mercuriale*, Martens (1999) found that 54.9% of oviposition bouts lasted less than 20 s and that females touching the substrate for up to 77 s laid no eggs. In *Platycnemis pennipes*, no eggs were laid when the abdomen was in contact with the substrate for less than 40 s (Martens 1992).

Explanations for oviposition behaviour without egg laying include the suggestion that egg flow stops during oviposition, that females are checking for the presence of predators near the stem (Rehfeldt 1990) or that some attempts at oviposition are unsuccessful (Martens 1992). Martens (1992) rejected the first explanation having found a constant increase in egg number laid with oviposition duration in *P. pennipes*. The proportion of *C. mercuriale* pairs affected by predators during oviposition behaviour was lower than that found for other odonates (Fincke 1986; Michiels & Dhondt 1990). Thus the presence of predators is unlikely to account for the termination of bouts before egg laying seen in many pairs. Thus, in *C. mercuriale*, non egg-laying bouts probably reflect unsuccessful attempts at oviposition due to the physical properties of the environment. When several bouts occurred on one stem, the female often terminated one short bout of oviposition by shifting her ovipositor relative to the stem before performing another oviposition bout. Often several rapid probing movements were performed with the ovipositor in a variety of positions until a 'suitable' position was found. The same movements were often performed after the last bout of oviposition on a stem before a pair flew to another stem. Such unsuccessful attempts at oviposition would necessitate the frequent changes between stems seen in *C. mercuriale*.

Females of *C. mercuriale* always submerged to oviposit up to their wings and submerged completely in 15.4% (6/39) of ovipositions. This behaviour has been observed in other populations of *C. mercuriale* (Martens 1999). It is found in a range

of endophytic dragonflies (e.g. Sawchyn & Gillott 1975; Fincke 1986) and may allow the female to avoid harassment by males during oviposition (Ubukata 1984; Waage 1987) or egg parasitoids that may have more difficulty locating eggs laid further below the water surface (A. Cordero Rivera pers. comm.). However, in *C. mercuriale*, where oviposition occurs in tandem, harassment of a pair by conspecific males rarely appeared to alter the female's behaviour. Submerged oviposition increases the area of suitable stem available to a female and allows more continuous bouts of oviposition to occur. Most importantly, it decreases the likelihood of desiccation of eggs as water levels drop during the summer (Fincke 1986). In *Enallagma hageni* (Walsh), Fincke (1986) found that no eggs hatched from exposed sedge stems whilst 96.3% of eggs hatched from submerged stems. Drying out of oviposition sites may constitute a significant risk to *C. mercuriale* since in the UK this species utilises shallow water bodies that are subject to considerable fluctuations in dimensions. Males of *C. mercuriale* have been reported (A. Cordero Rivera pers. comm.) to submerge completely with their mates in parts of the species range including in other parts of the UK.

The mean number of eggs laid by a pair during an oviposition was less than the mean initial clutch size of females elucidated in Purse & Thompson (2003b) which suggests that females do not lay their entire clutch of eggs in one visit to the breeding site. Since females have been observed ovipositing alone, they do not require a male to facilitate oviposition. The mean duration of continuous oviposition for a female was 11.2 min but varied considerably between females. This is shorter than oviposition duration in *Pyrrhosoma nymphula* (Sulzer) (Bennett & Mill 1995), in which females oviposited for 22.8 min, and may reflect the fact that females of *C. mercuriale* do not always lay their entire egg clutch in one oviposition episode.

There was a positive relationship between the total duration of oviposition on a stem and the number of eggs laid on it, for both stems where only one oviposition bout had been performed and for stems where several bouts of oviposition had occurred. Frequent non egg-laying bouts due to unsuccessful oviposition are probably partly responsible for the scatter around this relationship in *C. mercuriale*, especially for stems where more than one oviposition bout occurred. Similarly, Martens (1992) attributed large variation of egg deposition rate on a particular plant species in *P. pennipes* to the fact that actual oviposition proceeds discontinuously relative to oviposition behaviour.

The mean rate of egg deposition (14.06 ± 2.66 eggs per min) for *C. mercuriale* was among the highest noted for zygopterans. Other species with high mean deposition rate include *P. nymphula* (10.76 eggs per min, Bennett & Mill 1995) and *E. hageni* (13 eggs per min, Fincke 1986). However, most zygopterans deposit less than 10 eggs per min. Bick et al. (1976) found a range of 1.3 to 5.5 eggs deposited per min for five zygopteran species, *P. pennipes* deposited 6.4-9.4 eggs per min (Martens 1992) and *Enallagma cyathigerum* (Charpentier) deposited 4-8 eggs per min (Doerksen 1980). Oviposition rate varies between individuals of the same species due to the temperature of the water (Fincke 1986) and the suitability of the substrate (Waage 1978, Martens 1992). However differences between zygopteran species probably reflect the peculiarities of the study environment or measurement error rather than constituting major functional differences between species. For example, in *C. mercuriale* at Upper Crockford, the mean egg deposition rate was considerably higher than that of a French population of this species (3.66 or 5.08 eggs per min, Martens 1999).

Oviposition behaviour was unaffected by the presence of a cage, reflected in the similarity between caged and natural focals in a number of respects, the number and duration of bouts, the number of plant species used and the number of eggs laid. The similarity of oviposition behaviour in situations where conspecific males and other odonates were present (i.e. natural focals) and absent (i.e. caged focals) may indicate that these factors do not influence habitat selection for oviposition substantially. However, it is probable that pairs of *C. mercuriale* aggregate during oviposition. Martens (1999) found that pairs were attracted to oviposition sites containing other pairs over those that contained no pairs.

Hatching success, duration and mortality of hatch period

While *C. mercuriale* eggs can hatch in four weeks, a substantial proportion required a longer hatch period and hatching is not well synchronised within each clutch. In captivity, Corbet (1955) reported a shorter hatch period of 21 days. Levels of mortality during the hatch period were similar to those of another coenagrionid, *P. nymphula*, 25% failed to hatch versus 14% in this study but hatching was highly synchronised within clutches (Bennett & Mill 1995). The length of the hatch period of *C. mercuriale* was within the range for other species with direct (non diapause) egg development, i.e. between 5 and 40 days.

In addition to desiccation (Macan 1964; Fincke 1986; Bennett & Mill 1993), possible sources of egg mortality in endophytic species include infertility, unhatchability, predation, parasitism and over-use of stems (Waage 1978). The lack of previous clutches found in the stems before natural focals suggests stems are not over-used to a large extent in this population of *C. mercuriale* and thus eggs will not be susceptible to being punctured during repeated oviposition on the same stem. In addition, there was no evidence of predation on or parasitism of eggs whilst in stems. Thus the 14% mortality was attributed to infertility and unhatchability (cf. Bennett & Mill 1995). None of the stems used for natural focals was found exposed due to drops in water level so none of the eggs dissected had died due to desiccation.

Proximate cues for oviposition

Quantitative effects of continuous habitat variables on the duration of oviposition or the number of eggs laid could not be detected. There are a number of possible explanations for this. First, difficulties in tracking pairs and recovering stems resulted in a low sample size of stems. Second, these variables may be unimportant in determining the amount of oviposition that occurs once a pair has landed on a stem but may influence the decision of whether to land at all i.e. they act earlier in the hierarchy of decisions described by Wildermuth (1994) and Buchwald (1994a). Most of these factors could probably be assessed visually before landing and thus may constitute more general cues. For example, the fact that 43% of stems used for oviposition were within a patch containing only one stem suggests that plant stems can be detected visually before landing to a high degree of accuracy.

The time spent ovipositing having landed is most likely to be determined by the physical properties of the stem. Martens (1992) pointed out that the structure of water plants and their resulting usefulness to damselflies for oviposition varies greatly. With regards to the plant species used for oviposition by *C. mercuriale*, *Hypericum elodes*, *Potamogeton polygonifolius* and *Juncus articulatus* were used for oviposition more often than expected from their abundance whilst *Eleocharis palustris*,

Molinia caerulea and *Myrica gale* were used less often than expected. This is in contrast to the findings of Sternberg et al. (1999) where the use of plant species by *C. mercuriale* was not significantly different to the abundance of each species in the habitat and to the suggestion of Winsland (1997) that *C. mercuriale* oviposited in the submerged bases of rushes and sedges in Britain.

The plant species that were used more often for oviposition had similar physical properties. They were herbaceous and soft stemmed with thin cuticular layers that lacked collenchyma cells but had spongy parenchyma cells that may protect the eggs during development. Plant species that were used less often than expected for oviposition had strengthening layers in the stem that would impede the insertion of eggs. Strengthening layers took the form of a tough cambium layer in *Myrica gale*, a woody species, whilst in stems of *E. palustris* and *M. caerulea*, there was a subcuticular layer of collenchyma cells. Stems of *H. elodes*, *J. articulatus* and *P. polygonifolius* were also wider (at ca 2 mm) than those of other soft-stemmed herbs that were used rarely if at all for oviposition such as *Drosera rotundifolia* and *Anagallis tenella*. The stems and petioles of *Hypericum* and *Potamogeton* extend laterally under the water surface and have systems of side shoots which may facilitate their use for underwater oviposition in particular.

In several odonate species, larvae move from the oviposition site to a different sub-habitat having passed through the early larval instars (e.g. Corbet 1957; Macan 1964; Duffy 1994), and similarly *C. mercuriale* larvae may be capable of at least a limited degree of movement. Thus the proximate cues used for oviposition habitat selection at the final stage of the hierarchy may relate most closely to the habitat features required for egg development and efficient oviposition rather than indirectly indicating the permanence of habitat for larval development. The failure of eggs to hatch out of tougher stems has been cited as a possible cause of variation in egg mortality in another coenagrionid, *P. nymphula* (Bennett & Mill 1995), and illustrates the importance of physical stem properties.

Examples of physical properties of plants influencing oviposition behaviour can be found in other odonates. In *P. pennipes* the relative duration of oviposition on two plant species depended on their physical properties (Martens 1992). Short bouts of oviposition are permitted on *Myriophyllum spicatum*, the stems of which are narrow, irregularly shaped and interrupted by many filamentous leaves. Long bouts of oviposition are carried out on *Nuphar lutea* that has wider stalks without many leaves and thus presents a larger surface area for oviposition. In addition, plant surfaces covered by water-repelling layers such as the floating leaves of *Potamogeton natans* are probed but left unused by *P. pennipes*. Another example is where pairs of *Coenagrion pulchellum* (Vander Linden) oviposit preferentially in *Nuphar* and *Nymphaea* leaves that have holes in them made by the beetle *Donacia crassipes* over leaves that do not contain holes (Martens 1989) since such holes may provide better access to the fleshier leaf parts.

As well as using a range of plant species, *C. mercuriale* used a range of plant communities for oviposition at Upper Crockford including *Eleocharis palustris* swamp (S19, S19a, S19b); *Molinia caerulea* - *Potentilla erecta* mire (M25a), and *Hypericum elodes* - *Potamogeton polygonifolius* soakways (M29). Although some quadrats were found to be *Scirpus cespitosus* - *Erica tetralix* wet heath (M15, M15a), this community is not widespread in southern Britain and *Scirpus cespitosus* was not abundant on the site. Code numbers refer to Rodwell's (1991) National Vegetation Classification.

Many of the other habitat features used for oviposition at this small scale were consistent with broad scale features associated with adult populations in Britain and Europe. Oviposition behaviour occurred in unshaded, open areas of water without dense or tall emergent vegetation (cf. Sternberg et al. 1999) but with abundant submerged aquatic vegetation. At Upper Crockford, oviposition also occurred near to tussocks for shelter. Since *M. gale* is not used for oviposition, the higher percentage cover of this species near oviposition sites compared to general stream habitat also supports the suggestion that pairs require the shelter of tussocks for oviposition. Such shelter probably improves the accuracy of probing and oviposition movements and allows oviposition to proceed quickly. Oviposition occurred at a range of air temperatures, water temperatures and pH, in stems that were at least 1 mm in diameter and where only 4 cm of the stem protruded above the surface of the water.

Whilst in Europe oviposition occurred where water currents were between 0 and 0.05 m s^{-1} (Sternberg et al. 1999), in British chalk stream populations oviposition required discernible flow of not less than 0.029 m s^{-1} (A. Strange pers. comm.). In the heathland population in this study oviposition similarly occurred in shallow (usually less than 10 cm), unshaded, open areas in locations near to discernible flow (between 0.01 and 0.3 m s^{-1}). A requirement for flow has been found in other odonates (Wingfield Gibbons & Pain 1992; Siva-Jothy et al. 1995). The existence of flow preference has been attributed to a few factors. Siva-Jothy et al. (1995) found a reduction in the build up of algal matter on the surface of stems in fast flow. Such a build up could reduce the rate of oxygen supply to the eggs or prevent hatchlings from leaving the stem. Increased flow also reduces the boundary layer of still water around the egg, allowing oxygen to diffuse into it more rapidly and Halverson (1983) speculated that egg mortality and rate of embryogenesis in field populations of *Aeshna tuberculifera* Walker could be attributed to variation in dissolved oxygen concentrations in the field and thus to flow.

In conclusion, this investigation of the proximate cues used for habitat selection revealed several habitat features to be required by *C. mercuriale* for oviposition in the UK. Open, unshaded stream sections that are shallow and slow flowing with some areas of peat substrate are required. Such sections should contain abundant aquatic vegetation communities including both herbaceous, soft-stemmed, perennial (and evergreen) submergent or semi-emergent species for oviposition. Tussocks provide sheltered areas for oviposition. Features used at a small scale for these processes are consistent with those that determine the distribution of adults within and between sites.

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